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Original Article

Propagation strategy shapes citrus growth and molecular traits across swamp and upland agroecosystems

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Abstract

Previous studies have demonstrated drought-induced physiological and molecular responses in citrus under controlled environments; however, whether these stress-response pathways operate predictably across contrasting field agroecosystems and under different propagation strategies remains unclear. This study aimed to determine how propagation strategy modulates citrus growth performance, leaf anatomy, stress-related gene expression, and untargeted metabolomic profiles across swamp and upland agroecosystems during early field establishment. Two different citrus species (*Citrus nobilis* L. cv. Pontianak Siam and *C. reticulata* Blanco cv. Keprok RGL) were established under open-field conditions in upland mineral soil and tidal-swamp acid-sulphate soil (pH 4.2–5.2) in East Kalimantan, Indonesia. The propagation techniques used were conventional chip budding and bud-assisted marcotting, locally known as 'Okucang'. The environmental background was the primary driver of variation. In the soil PCA, the two systems (upland and swamp) were separated along PC1 (72.3% variance). Siam Pontianak, propagated through bud-assisted marcotting, produced significantly taller plants than other treatments, especially under upland conditions ($P < 0.05$). In contrast, Siam Pontianak propagated through chip budding showed a wider canopy in upland areas. Canopy development ($P < 0.05$) decreased under swamp conditions. Regardless of variety or propagation method, palisade mesophyll thickness was thicker in swamp areas than in upland areas. Secretory gland diameter was comparable between environments but tended to decrease under swamp conditions. Leaf Fe and Mn content were dominantly accumulated in the swamp area, whereas upland conditions favoured Ca–K enrichment and structural growth. Stress-responsive genes, Osmotin and aquaporins (*PIP1/PIP2*), were significantly upregulated under swamp conditions ($P < 0.05$), but their regulation varied with soil redox chemistry and propagation method. Metabolomic profiling additionally discriminated among agroecosystems (PLS-DA PC1 = 32.7%), with terpenoid- and antioxidant-related metabolites (VIP ≥ 1.2). Propagation effects were environment-dependent, reflecting genotype × environment × propagation interactions: bud-assisted marcotting enhanced stress-associated traits under swamp conditions, whereas chip budding promoted structural growth under upland conditions. These results extend previous controlled-drought experiments by demonstrating how established stress-response pathways operate within lowland agroecosystems shaped by soil mineral chemistry and propagation architecture.

Keywords: Citrus, Gene expression, Metabolomics, Propagation method, Swampland

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Introduction

Citrus production in tropical regions is increasingly challenged by the expansion of cultivation into marginal environments, driven by climate variability and intensified land-use competition. Among these environments, strong contrasts exist between upland and swamp or tidal lowland agroecosystems, which differ fundamentally in hydrological regime, soil physical characteristics, and nutrient dynamics. These differences are particularly significant in many parts of Southeast Asia, including Indonesia, where large tracts of swamp and tidal lands with potential for tree crops remain underutilized despite their importance as a strategic resource base for future agricultural production.

Generally, upland landscapes have higher relief, well-drained soils, and are primarily aerobic. Under such circumstances, organic matter decomposition is high, and nutrient mineralization is active, particularly of nitrogen and phosphorus, making these nutrients more available to plants (Brady and Weil, 2017). Water balance in upland ecosystems is primarily controlled by the vertical redistribution of rainfall via infiltration and percolation, resulting in cycling between wet and dry events that governs soil water availability and crop production. Upland, or dryland, covers about 1,444,732 km² of land area (±75.6% of Indonesia's national land area) and is dominated by mineral soils such as Ultisols and Inceptisols. These soils are frequently acidic and often have shallow, efficient depths, but can support good agricultural productivity when managed with proper liming, organic inputs, and suitable erosion-control practices (Hikmat et al., 2022). However, intermittent drought, uneven rainfall distribution, and limited water availability continue to pose serious constraints on the cultivation of perennial fruit crops (citrus) in upland agroecosystems.

By contrast, swamps in Indonesia are waterlogged, with regular or occasional soil saturation, creating anaerobic or hypoxic conditions that strongly affect the underlying biogeochemical processes. A major limitation of swamp soils, particularly tidal and acid sulphate soils, is extreme soil acidity and the presence of phytotoxic elements such as soluble iron and aluminium. Flooding–drainage cycles in these environments trigger rapid redox-driven transformations that can cause sharp declines in soil pH and elevated concentrations of Fe^{2+} and Al^{3+}, severely restricting root growth, nutrient uptake, and overall crop performance (Fahmi et al., 2018; Sulaeman et al., 2024). Swamp and peat soils are also very sensitive to drainage or land-use changes, but they typically lack the essential macro- and micronutrients essential for plant growth. Although total swampland in Indonesia is estimated at 32.64–33.39 million ha, only 14–19 million ha are potentially suitable for agricultural land, and less than 7 million ha have been effectively developed (Noor et al., 2022).

Citrus exhibits substantial plasticity in hydraulic regulation and stomatal behaviour under drought or waterlogging, mediated through hormonal signalling involving abscisic acid (ABA), ethylene, and oxygen sensing (Buckley, 2019; Li et al., 2024). Key stress-responsive genes, such as *ACS2* (ethylene production), *CsNF-YA5* (ABA-dependent transcription factor), *Osmotin* (osmotic protection), and aquaporins (*PIP1*/*PIP2*), coordinate responses to water-mediated challenges (Yaneff et al., 2015; Yepes-Molina et al., 2020; Dahro et al., 2023). In particular, secondary metabolites such as flavonoids and terpenoids help buffer oxidative stress and stabilize membranes under unfavourable conditions (Zandalinas et al., 2017; Balfagón et al., 2025).

This situation is particularly evident in tropical countries such as Indonesia, where citrus production occurs across heterogeneous landscapes ranging from swamp lowlands to upland systems. Controlled-environment studies have revealed some of the mechanisms underlying drought responsiveness in citrus (Devy et al., 2025), but information on how soil–water regimes, propagation methods, and genotypes interact to generate coordinated morphological, physiological, and molecular responses under actual agroecosystem conditions is lacking. As a result, the genetic and epigenetic bases for how citrus adapt across contrasting swamp–upland environments are poorly understood, limiting the development of site-specific planting and management interventions. While Devy et al. (2025) documented baseline stress-responsive gene activation in a high-elevation shade-house environment, the current work investigates the conservation vs. amplification of these molecular responses and the eco-reprogramming for contrasting lowland field agroecosystems marked by different soil hydrology and propagation structures. Building on the earlier framework established for controlled environments, we explored how soil mineral chemistry and propagation architecture conditioned stress-response pathways in a genotype × environment × propagation (G×E×P) context through interactions among them and genotype under heterogeneous conditions.

The direct consequences of short- and long-term root zone waterlogging are clearly detailed in experimental work with citrus rootstocks, where both treatments have a major impact on conducting capacity, stomatal conductance, photosynthesis rate, and biomass production, with these effects highly genotype/root system-dependent (Pérez-Jiménez and Pérez-Tornero, 2021; He et al., 2023). Comparable responses are also observed in other tropical fruit crops, including mango, guava, and jackfruit, unless plants possess adaptive traits, such as adventitious root formation or aerenchyma development (Fischer et al., 2023). The manner of vegetative propagation is a relatively unexplored factor influencing citrus behavior under marginal conditions. Reproductive methods (e.g., budding and bud-assisted marcotting) generate distinct root system architectures, vascular connections, and hydraulic properties that influence water uptake, nutrient acquisition, and stress-related signaling during the juvenile vegetative stage (Singh, 2018; Habibi et al., 2022; Albrecht et al., 2017; Albrecht et al., 2020). Recent developments in multi-omics technologies, including transcriptomics, metabolomics, ionomics, and phenotypic traits, are effective tools for deciphering these intricate interactions (Misra et al., 2019; Yang et al., 2025). Nonetheless, integrated multi-omics analyses of citrus grown under contrasting upland and swamp agroecosystems in tropical field environments remain scarce.

Thus, while certain stress-responsive genes and physiological indicators have been previously validated under controlled drought conditions, it remains unclear whether these molecular pathways exhibit ecological stability or context-dependent modulation when citrus is grown in lowland open-field systems with acid sulfate soils and propagation-driven architectural differences. Filling this information gap is critical to understanding whether control-environment stress responses can be translated into field-based management strategies.

To date, few studies have simultaneously evaluated how soil–water regimes, propagation strategies, and genotype interact to shape coordinated morphological, physiological, and molecular responses in young citrus trees grown under real agroecosystem conditions. The mechanisms by which citrus trees adapt to swamp and upland environments remain unclear, thereby hampering the development of practical propagation and cultivation protocols.

In this study, we investigated young citrus trees derived from budding and budding-assisted marcotting grown under contrasting upland and swamp agroecosystems in East Kalimantan, Indonesia. Using two citrus species, an integrated multi-omics framework combining morphological and anatomical assessments with ionomic, transcriptomic, and metabolomic analyses was observed. In particular, the objective of this study was to (i) describe growth and physiological distinctions between upland- and swamp-grown citrus trees, (ii) identify how propagation method affects root–shoot coordination and stress-responsive molecular pathways, and (iii) discover core metabolic or transcriptional signatures that are related to improved tolerance against waterlogged as well as chemically restricted soils.

By explicitly integrating genotype × environment × propagation (G×E×P) interactions across contrasting lowland systems, this study extends prior controlled drought research toward an agroecosystem-scale understanding of citrus adaptation.

Compared with our previous controlled-environment study (Devy et al., 2025), which primarily evaluated drought-duration effects under uniform soil conditions, the present study demonstrates that soil mineral chemistry and propagation architecture become dominant drivers of citrus stress responses under heterogeneous lowland agroecosystems.

Material and Methods

Experimental site, plant materials and cultural practices

The field experiment was conducted from January to December 2024 in Paser Regency, East Kalimantan, Indonesia (00°49′S; 116°24′E; approximately 10 m above sea level). Two different agroecosystem locations were chosen in order to represent contrasting soil and water management situations found commonly in Indonesian citrus cultivation: (1) an upland location (L1), with free draining, non-flooded acid mineral soil with no risk of waterlogging; and (2) a tidal swamp land location (L2), having potential acid sulphate soils subject to periods of tidal flooding, and containing pyritic subsoil layers that release elevated concentrations of soluble Fe^{2+} and Al^{3+} under oxidative conditions. It was characterized by poor nutrient conditions and an acidic soil pH of 4.2-5.2 (Ritung et al., 2011; Kunarso et al., 2022).

The plant material consisted of two commercial citrus genotypes: *Citrus nobilis* cv. Siam Pontianak (V1) and *C. reticulata* cv. Keprok RGL (Rimau Gerga Lebong) (V2). Propagules of these cultivars were propagated by two propagation procedures, namely (1) Conventional chip budding technique (P1) and (2) Bud-assisted marcotting or 'Okucang' method of propagating tree crops (P2), as done according to Amali et al. (2001). They were all grafted onto C. *jambhiri* cv. JC rootstock, frequently utilized in Indonesia (Yulianti et al., 2021; Devy et al., 2024).

Trees were spaced at 3 m × 3 m (1,111 trees ha^{-1}). The soil was enriched with 25 kg of well-composted goat manure and one kilogram of agricultural lime ($CaCO_3$) in each planting hole (60 cm x 60 cm x 60 cm), so as to reduce soil acidity and provide initial nutrients. Routine cultural practices, such as weeding and pest surveillance, were performed equally across all treatments.

Experimental design

The study was conducted in two contrasting agroecosystems, namely swamp and upland areas, to evaluate the effects of propagation methods and citrus species on early plant performance. At each location (swamp and upland), the experiment was conducted on a factorial randomized complete block design with two factors: propagation method (budding and marcotting) and citrus species (*C. nobilis* cv. Siam Pontianak and *C. reticulata* cv. Keprok RGL), with three replications. A two-way ANOVA was performed to evaluate treatment effects within each location, and differences between locations were analysed using independent *t*-tests.

Soil and tissue elemental analysis

Composite soil samples (0–30 cm depth) were collected from each experimental block at the beginning of the study. Soil samples were air-dried, ground, and passed through a 2-mm sieve prior to analysis. Soil pH was determined in a 1:2.5 soil-to-water suspension using a calibrated pH meter. Soil organic carbon was measured using the Walkley-Black wet oxidation method. Available phosphorus was extracted using the Bray-1 method and quantified spectrophotometrically. Exchangeable cations (Ca^{2+}, Mg^{2+}, K^{+}, Na^{+}) were extracted with 1 M ammonium acetate (pH 7.0) and analysed by atomic absorption spectrophotometry (Nelson and Sommers, 1996; Öhlinger et al., 1996).

Content of total elements in samples (soil and leaf tissue) was measured by energy dispersive X-ray fluorescence spectrometry (ED-XRF; Malvern Panalytical Epsilon 4), calibrated using certified standards (NIST 2709a for soil and NIST 1515 for plant tissue). XRF analysis was used to measure the concentration of the elements Na, Mg, Al, Si, P, S, K Ca Mn Fe Zn and Sr. Leaf tissue was sampled 6 months after planting the crop, and whole mature but fully expanded leaves were washed using deionized water, oven-dried at 65°C for 72 h, and pulverized to pass through a 0.5-mm sieve before XRF analysis (Hanlon et al., 2012).

Vegetative growth measurements

Vegetative growth parameters were evaluated at 12 months after field planting. Plant height was measured from the soil surface to the apex of the main stem using a metric tape. Canopy volume was estimated from canopy width measurements taken along two perpendicular axes (north-south and east-west directions) at the widest point.

Leaf anatomical analysis

Leaf anatomical characteristics were examined using standard microtechnique procedures (Metusala, 2017). Fully expanded leaves from the middle portion of current-season spring flush shoots were collected and immediately fixed in FAA solution (formalin-acetic acid-ethanol, 5:5:90 v/v/v) for 48 h. Fixed tissues were dehydrated through a graded ethanol series (50%, 70%, 85%, 95%, and 100%), cleared in xylene, and infiltrated with paraffin wax (melting point 56–58°C). Cross-sections (10–12 μm thickness) were cut using a rotary microtome (Leica RM2125 RTS) and mounted on glass slides. Anatomical measurements included: palisade mesophyll thickness, spongy mesophyll thickness, and oil gland diameter. For each treatment combination, three leaves per plant (from three replicate plants) were analysed, with five microscopic fields per leaf examined (n = 45 observations per treatment).

Stomatal density measurements were conducted on nail polish imprints of the abaxial leaf epidermis. Leaves were freshly sampled in the morning, and nail polish was applied to the abaxial leaf surface. The impressions were subsequently detached and mounted on glass slides after drying. Stomata were observed in five random fields per leaf (0.015 mm^2 of area for each field) at 400× magnification, and stomatal density was indicated as the number of stomata per mm^2 (Auliya et al., 2019).

Gene expression analysis

A composite-sampling strategy was used to estimate cultivar-level gene expression under field conditions. Fifteen plants from each cultivar were collected across the four sampled agroecosystems, and fully expanded leaves at the early stage were bulked to obtain one biological composite sample per treatment.

RNA from all samples was isolated using the RNeasy Plant Mini Kit (Qiagen), followed by reverse transcription with the iScript cDNA Synthesis Kit (Bio-Rad). Real-time quantitative PCR analysis of seven stress-responsive genes (*ACS2*, *ETR1*, *CHLASE*, *CsNF-YA5*, *Osmotin*, *PIP1*, and *PIP2*) (Table 1) relative to EF1 as an internal control was performed using primer pairs previously validated in citrus by Yulianti et al. (2021) and used in our previous controlled-environment study (Devy et al., 2025). The same primer sequences were used here to enable direct comparison between controlled drought responses and field agroecosystem conditions.

As described in Yulianti et al. (2021), the study was conducted using a Mini Opticon Real-Time PCR System (Bio-Rad). The relative quantification was done ($2^{-\Delta\Delta Ct}$ method) according to the analytical method described by Devy et al. (2025), as our controlled-environment study, but here applied to a contrasting lowland field environment.

Gene expression analysis was performed using one composite biological sample per treatment–environment combination. For each composite sample, young leaves from fifteen plants were pooled to represent cultivar-level transcriptional responses within each agroecosystem and propagation treatment. This composite sampling approach was used to capture population-level transcriptional trends under field conditions while minimizing plant-to-plant variability. Gene expression was quantified once per composite sample without technical replicates; therefore, expression profiles are interpreted descriptively to illustrate relative modulation patterns rather than statistically validated fold changes.

Table-1. Primer sequences used for qPCR analysis (Yulianti et al., 2021).

Gene	Forward primer (5'→3') Reverse primer (5'→3')	Amplicon size (bp)	GenBank Accession	Primer efficiency (%)	Correlation coefficients (R^2)
Osmotin	FW: *CAACGACCTCTCCTCCTACG* RV: *ACAGCTCGTCCGTACCAAAC*	218	MK123456	97.2	0.98
PIP1	FW: *AGGATTACACGGAGCCACCT* RV: *TGCTTTTGGATTTGGACACG*	156	XM_006478921	92	0.91
PIP2	FW: *TGTGTTCATGGTTCACTTGG* RV: *TGAATGGTCCAACCCAGAAG*	137	XM_006476603	90.2	0.97
CHLASE	FW: *ACCGCTTGTGCACCTGAAG* RV: *TGTCCATGTGACCATAATCTGTAGC*	103	NM_001330707	91.9	0.98
ACS2	FW: *CACAGTGTTCACCAAGGAGTC* RV: *CGAGTAAATGATACCGACCCTAA*	148	XM_006464243	93.4	0.98
ETR1	FW: *CAGGAGAGAAGCGGAAACAG* RV: *GCTCGGGTGTCAATTCAGTC*	134	XM_006465551	98	0.96
CsNF-YA5	FW: *CATTTCAGAATGGGGAAATCAT* RV: *TTCTTCCTCATCTCACCCAAAG*	99	XM_006468604	94.4	0.96
EF1	FW: *ATTGACAAGCGTGTGATTGAGC* RV: *TCCACAAGGCAATATCAATGGTA*	136	AY498567.1	98.1	0.94

Leaf untargeted metabolite profiling

Each leaf metabolomic sample was a composite of leaf tissue pooled from 15 trees per treatment across areas. Crude ethanol extract of dried leaves was analysed by GC-MS (HP-5MS UI column; 1 µL injection, 10:1 split, He carrier, ion source 230°C, m/z 50-600, 60-280°C gradient). Metabolites were identified by comparison with the NIST14 library. Data were processed with MS-DIAL and analysed with MetaboAnalyst 6.0 after log transformation and autoscaling using PCA, PLS-DA, and hierarchical clustering. KEGG reference-enabled pathway enrichment. PLS-DA was internally validated by using R^2, Q^2, and a 200-permutation test. Metabolites with VIP ≥ 1 were considered as potential discriminant features. Because GC–MS was used to obtain qualitative, untargeted metabolite profiling, no calibration curve was applied in this method (Fiehn, 2017; Wishart, 2019; Salem et al., 2020; Abadie et al., 2022). The metabolomic results should be interpreted as exploratory and hypothesis-generating, revealing pathway-level metabolic differences rather than absolute quantitative differences.

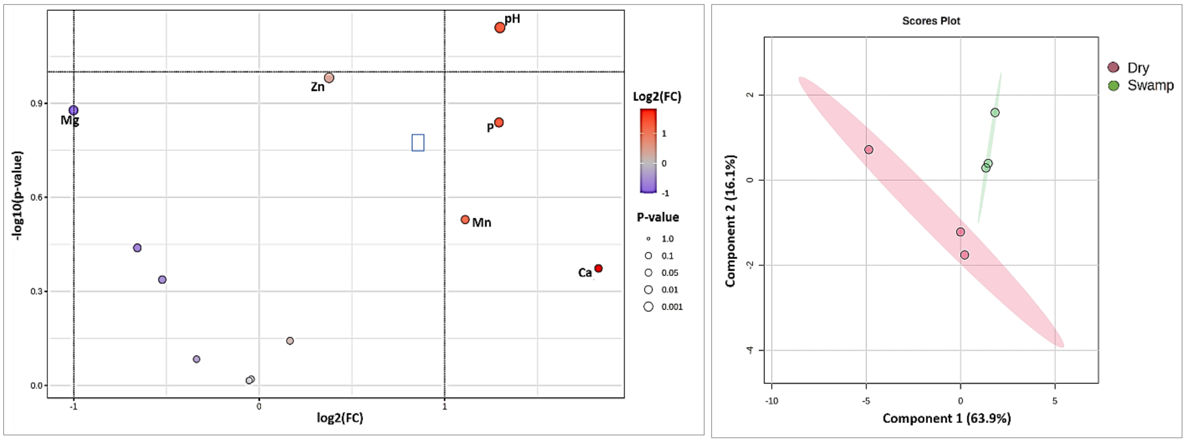
Statistical analysis

The data were analysed using MINITAB 16 (Minitab Inc., USA), and statistical differences ($p < 0.05$; $p < 0.01$) were assessed using Tukey's Honestly Significant Difference (HSD) test. For metabolomic data, heatmaps and hierarchical clustering were produced in R 4.3.2 using the *pheatmap* package after standardizing variables via Z-scores. Gene expression data were transformed using log10-transformed [log10(expression + 1)] to reduce skewness for heatmap visualization. Principal Component Analysis (PCA) was conducted using the MASS, *ggplot2*, and *factoextra* packages in R. MetaboAnalyst was used to visualize the metabolomic pathway.

Results

Soil physicochemical contrasts define environmental gradients

Soil elemental composition differed markedly between upland and swamp sites (Figure 1). Log_2 fold-change analysis showed that several major and trace elements varied by more than ±1.0 $\log_2$ units relative to the site mean, corresponding to ≥2-fold differences between environments (Figure 1a). Principal component analysis separated the upland (L1) and swamp (L2) soils along PC1, which explained 72.3% of the total variance (Figure 1b). No overlap between sites was observed despite replication (n = 3 composite samples per site), indicating strong and consistent environmental differentiation in soil physicochemical properties.

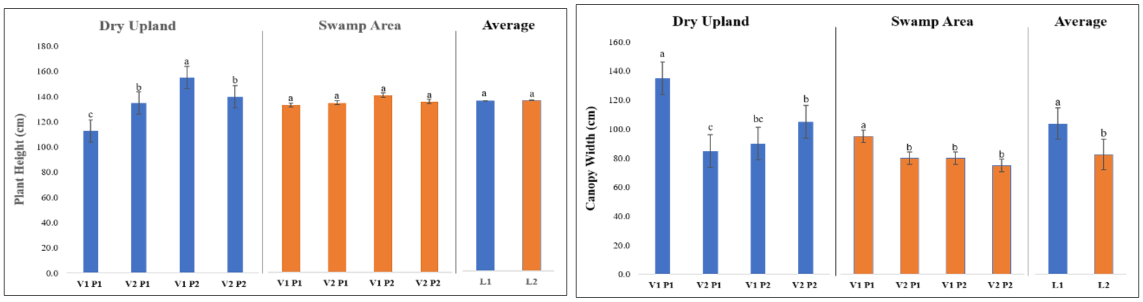


(a) (b)

Figure-1. Soil physicochemical characterization showing environmental contrasts between upland and swamp sites. (a) Fold-change of major and trace elements (values represent $\log_2$ fold-change relative to site mean); (b) Principal component analysis (PCA) biplot showing clear multivariate separation of upland (L1, red circles) and swamp (L2, green circles) soil samples along PC1 (72.3% variance). Each point represents a composite soil sample (n = 3 per site).

Environment and propagation shape growth architecture

Siam Pontianak, propagated through bud-assisted marcotting, produced significantly taller plants than other treatments, especially under upland conditions ($P < 0.05$). However, it did not show a difference in swamp areas. Similarly, there was no difference between locations (Figure 2a). In contrast, Siam Pontianak propagated through chip budding showed a wider canopy in upland areas. Interestingly, the same results occurred under swamp-area conditions (Figure 2b). Canopy width was significantly greater under upland conditions than under swamp conditions ($P < 0.05$).



(a) **(b)**

Figure-2. Morphological responses of citrus varieties to environment and propagation method. (a) Plant height (cm) and (b) canopy width (cm) of Siam Pontianak (V1) and Keprok RGL (V2) propagated by chip budding (P1) or bud-marcotting (P2) under upland (L1) and swamp (L2) conditions. Bars represent mean ± SD (n = 4 plants per treatment). Different lowercase letters indicate significant differences among treatments within each environment (Tukey's HSD, $P < 0.05$).

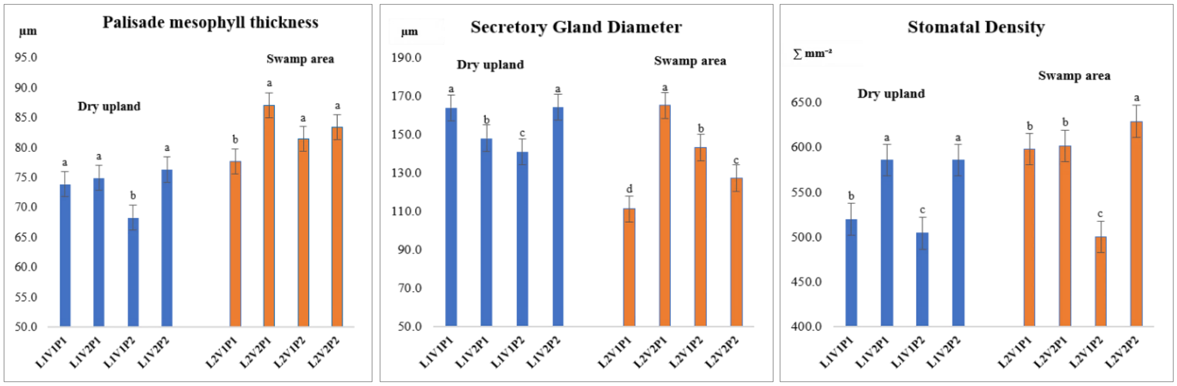
Leaf anatomical plasticity reflects environmental control

Leaf anatomical traits showed significant quantitative variation among treatments (Figure 3). The combination of variety and propagation method shows a significant difference in palisade mesophyll thickness across locations ($P < 0.05$; Figure 3a). In upland conditions, Siam Pontianak propagated by bud-assisted marcotting has a thinner palisade compared to other treatments. Meanwhile, in swamp areas, Siam Pontianak propagated by chip budding has a thinner mesophyll palisade. Furthermore, regardless of variety or propagation method, palisade mesophyll thickness was thicker in swamp areas than in upland areas. The secretory gland diameter in Siam Pontianak propagated through both chip budding and bud-assisted marcotting was significantly larger in upland conditions, whereas in swamp areas, Keprok RGL actually showed a larger secretory gland diameter ($P < 0.05$; Figure 3b). In addition, secretory gland diameter was comparable between environments but tended to decrease under swamp conditions. There were significant differences in stomatal density between the genotypes. It seems that Keprok (RGL) was consistently higher than that of Siam Pontianak across all environments and propagation methods ($P < 0.05$; Figure 3c). Measurements were performed in 5 microscopic fields per plant (n = 4 plants per treatment).

Leaf mineral profiles reveal ion uptake strategies

The normality of leaf mineral data was examined and, where applicable, the data were transformed prior to analysis. Variables with zero-inflated distribution (Mg, S, Si, and Na) were $\log_{10}(x + 1)$-transformed to achieve normality and homoscedasticity; for K, Ca, P, Fe, Mn, Zn, Cu, Br, and Sr, $\log_{10}$-transformation was applied only.

Environmental conditions strongly shaped leaf mineral composition, with both general and element-specific differences between upland (L1) and swamp (L2) sites (Table 2). Among the nutrients analysed, Fe and Mn showed the strongest location-dependent variation, with consistently higher concentrations in swamp-grown plants regardless of genotype or propagation method. Such patterns are consistent with flooding-induced disruption of Fe uptake and root regulatory responses in citrus (Martínez-Cuenca et al., 2015). In contrast, Ca and K were generally more abundant under upland conditions, reflecting differences in soil chemical availability and redox conditions between the two environments.



a **b** **c**

Figure-3. Leaf anatomical responses to environment, genotype, and propagation method. (a) Palisade mesophyll thickness (µm), showing strong environmental effect with swamp > upland ($p < 0.05$); (b) Secretory gland diameter (µm), showing reduction under swamp conditions ($p < 0.05$); (c) Stomatal density (stomata mm^{-2}), showing genotype dominance with RGL > Siam ($p < 0.05$). Bars represent mean ± SD (n = 4 plants, five microscope fields per plant). V1 = Siam Pontianak; V2 = Keprok RGL; P1 = chip budding; P2 = bud-marcotting; L1 = upland; L2 = swamp. Different lowercase letters indicate significant differences among all eight treatment combinations (Tukey's HSD, P < 0.05).

Table-2. Leaf mineral content (ppm, dry weight) across treatments.

Treatments	Fe	Mn	Ca	K
V1 P1 L1	0.1±0.0^{b}	425.9±94.1^{ab}	43.5±8.8^{ab}	13.0±2.9^{ns}
V1 P2 L1	0.1±0.0^{b}	391.4±28.2^{ab}	36.9±10.4^{ab}	23.2±2.9
V2 P1 L1	0.2±0.0^{b}	318.8±68.1^{b}	27.7±6.0^{b}	23.6±9.6
V2 P2 L1	577.9±500.9^{ab}	319.6±70.9^{b}	30.6±3.3^{ab}	11.9±5.5
V1 P1 L2	796.0±89.4^{a}	403.9±107.0^{ab}	53.3±6.4^{a}	11.2±2.2
V1 P2 L2	939.7±69.6^{a}	432.5±94.3^{ab}	52.5±3.4^{a}	10.0±0.8
V2 P1 L2	0.1±0.0^{b}	511.3±40.5^{a}	41.6±3.8^{ab}	17.8±4.2
V2 P2 L2	317.2±549.2^{ab}	501.7±14.8^{a}	42.3±9.1^{ab}	15.6±5.9

V1=Siam Pontianak; V2=Keprok RGL; P1=chip budding; P2=bud-marcotting; L1=upland; L2=swamp. Values are mean±SD (n=4). Different letters indicate significant differences (Tukey's HSD, $p<0.05$), ns= non-significant.

There were no consistent or significant differences in the macro-elements P, S, and Mg across locations and treatments (non-significant ANOVA and overlapping Tukey groupings). Zn and Cu also did not show consistent patterns across locations, although their concentrations varied among treatments. There was actually little silicon and sodium in either the cases or the controls, precluding meaningful comparisons of treatments.

The propagation treatment alone did not have a significant or consistent effect on leaf mineral composition; the observed differences were context-specific and moderated by environmental × genotype interactions rather than by any general effect of the propagation method. Genotype-associated differences were limited and element-specific. Mn and Sr exhibited significant genotype-related variation under swamp conditions, with Keprok RGL generally showing higher Mn and Sr concentrations than Siam Pontianak.

In contrast, Fe and Ca did not exhibit consistent main effects by genotype. Overall, these results indicate that environmental control was the dominant factor shaping leaf mineral profiles during early citrus establishment, with secondary and element-specific modulation by genotype and limited, context-dependent effects of propagation method.

Stress-responsive gene expression reveals environment- and propagation-dependent modulation

Relative transcript abundance ($2^{-\Delta\Delta Ct}$) of seven stress-related genes varied significantly among environments, genotypes, and propagation methods ($P < 0.05$; Figure 4).

Consistent with previously reported controlled drought responses (Devy et al., 2025), *Osmotin* and aquaporin genes (*PIP1* and *PIP2*) exhibited elevated expression under stress-associated swamp conditions relative to upland environments (Figure 4d–f). These patterns confirm that canonical osmotic adjustment and hydraulic regulation pathways remain responsive under lowland field agroecosystems.

The gene expression analysis revealed that the expression levels of *ACS2* and *ETR1* were also significantly different under upland and swamp conditions (Figure 4a–b), suggesting environment-dependent modulation of ethylene-related signaling. Distinct environment-dependent variation in transcript abundance was observed for *CHLASE* (Figure 4c), indicating differing chlorophyll metabolism under diverging soil–water conditions.

The propagation method also affected the magnitude of gene-expression responses, most notably in PIP1 and PIP2 (Figure 4e-f), implicating root system architecture as a modulator of stress signaling under field conditions. Variation in *CsNF-YA5* expression between genotypes (Fig. 4 g) highlighted transcriptional plasticity at the cultivar level

Taken together, these results show that the core pathways of stress response identified under controlled conditions remain active, but their expression patterns are modulated by agroecological context and propagation architecture (genotype×environment×propagation (G×E×P) interactions under field conditions).

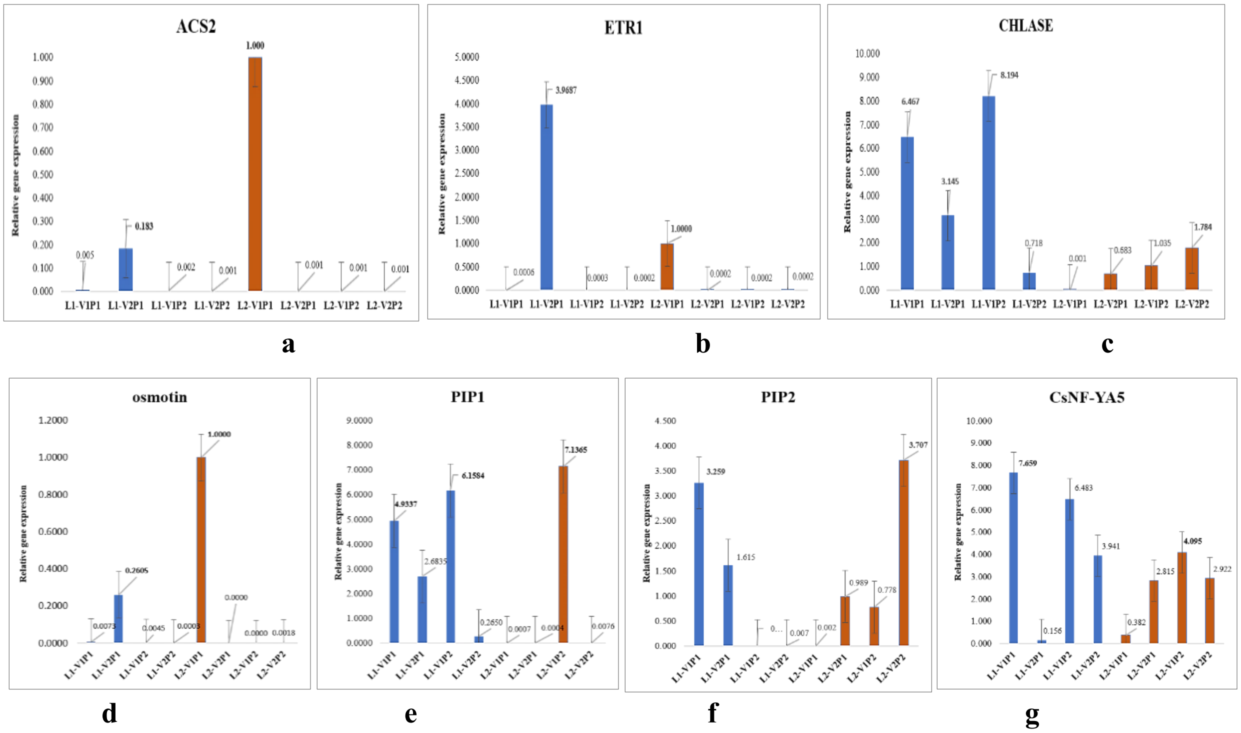


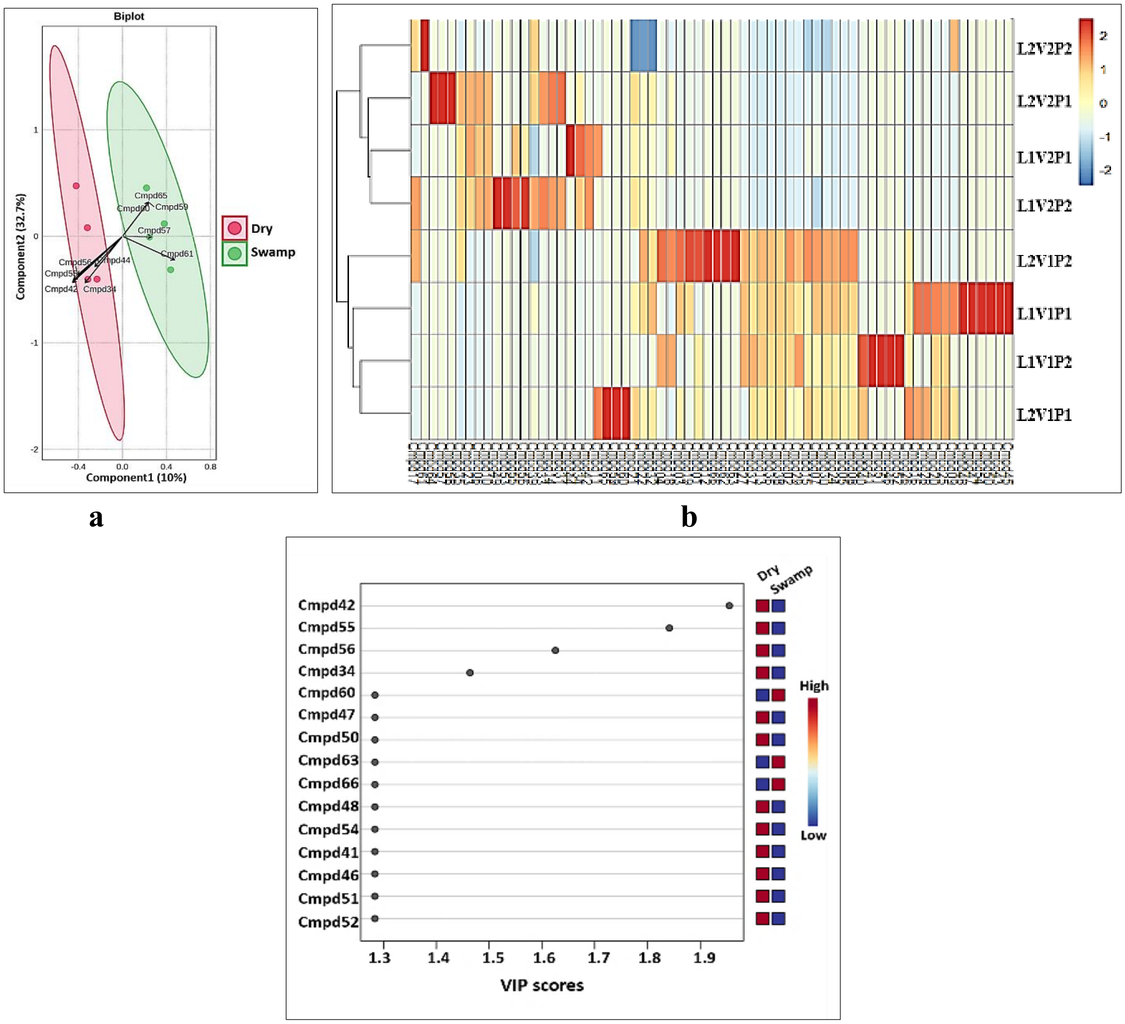
Figure-4. Environment-, genotype-, and propagation-dependent expression of stress-responsive genes in citrus scions.

Relative transcript abundance ($2^{-\Delta\Delta Ct}$) of (a) *ACS2*, (b) *ETR1*, (c) *CHLASE*, (d) *Osmotin*, (e) *PIP1,* (f) *PIP2*, and (g) *CsNF-YA5* in Siam Pontianak (V1) and Keprok RGL (V2) propagated by chip budding (P1) or bud-marcotting (P2) under upland (L1) and swamp (L2) conditions. Relative expression ($2^{-\Delta\Delta Ct}$) values are shown for one composite biological sample per treatment per environment. Values represent relative transcriptional modulation patterns.

However, unlike the controlled drought system described previously, the present field experiment indicates that transcriptional responses are strongly modulated by soil redox chemistry and propagation architecture.

Metabolomic signatures distinguish environmental adaptations

Partial least squares discriminant analysis (PLS-DA) showed a clear separation between the upland and swamp samples along PC1 (32.7% of overall variance), while PC2 (10.0%) mainly represented the genotype difference in that species population structure (Fig. 5a). Hierarchical clustering grouped the samples by genotype mainly, with secondary grouping by environment and the way they were propagated (Figure 5b). Variable importance in projection (VIP) analysis identified discriminant metabolites with VIP scores ≥ 1.2 as pivotal compounds involved in environmental separation (Fig. 5c), including those that showed differential accumulation between upland and swamp conditions. Among them, representative compounds are listed in Table 3.



a b

c

Figure-5. (a) The PLS-DA explains 32.7% of the variance in PC1 (mainly representing environmental variation) and 10% in PC2 (capturing genotype). (b) Hierarchical clustering analysis indicates that metabolomic organization is primarily driven by genotype differences, with subsequent grouping driven by environment and propagation method. (c) VIP analysis of metabolites responsible for environmental discrimination (VIP ≥ 1.2), colour intensity reflecting the relative accumulation under upland and swamp conditions.

Table-3. Key discriminant metabolites (VIP>1.2) showing environment-specific accumulation.

No.	Compound code	Putative metabolite name	Chemical class	VIP score	Enriched environment
1	Cmpd55	Melezitose	Carbohydrate derivative	1.85	Upland (Dry)
2	Cmpd56	Tau-cadinol acetate	Monoterpenoid alcohol	1.72	Upland (Dry)
3	Cmpd34	Vitamin E–like compound	Terpenoid antioxidant	1.61	Upland (Dry)
4	Cmpd60	Diterpenoid alcohol derivative	Diterpenoid	1.53	Upland (Dry)
5	Cmpd48	Polymethoxylated benzopyran derivative	Phenolic-terpenoid	1.36	Upland (Dry)
6	Cmpd47	Sesquiterpenoid oxide derivative	Sesquiterpenoid	1.48	Swamp
7	Cmpd50	Methyl docosatetraenoate	Fatty acid ester	1.45	Swamp
8	Cmpd63	Nootkatone-11,12-epoxide	Oxygenated sesquiterpenoid	1.41	Swamp
9	Cmpd66	α-Guaiene	Sesquiterpene hydrocarbon	1.39	Swamp
10	Cmpd54	Columbin	Diterpenoid	1.34	Swamp
11	Cmpd41	Heptadecenoic acid methyl ester	Fatty acid ester	1.33	Swamp
12	Cmpd46	Isopropyl-octahydronaphthalene derivative	Sesquiterpenoid	1.31	Swamp
13	Cmpd51	6-epi-shyobunol	Sesquiterpenoid alcohol	1.29	Swamp
14	Cmpd52	α-Acorenol	Monoterpenoid oxide	1.27	Swamp

Discussion

This study outlines the following main results: the environmental water regime determines variation in all measured responses (Figures 1–5; Tables 2, 3); propagation influences stress responses in an environment-dependent manner (Figures 2–4); and genotype-specific strategies further shape adaptive patterns (Figures 3–5). Importantly, these findings confirm previously reported drought-responsive pathways (Devy et al., 2025), while demonstrating that soil mineral chemistry and propagation architecture act as dominant regulators of stress-response coordination under heterogeneous field conditions.

In this study, differences between upland and swamp agroecosystems in East Kalimantan are characterized by fundamentally distinct hydrological regimes, resulting in contrasting soil chemical landscapes and systemic plant responses at morphological, physiological, ionic, transcriptomic, and metabolic levels. In agreement with former multi-omics studies (Zandalinas et al., 2022; Rao et al., 2023), the environmental water regime emerged as the major contributing factor, explaining >50% of overall variation in ionomic (Table 2), transcriptional (Figure 4), metabolomic (Figure 5, Table 3), anatomical (Figure 3), and morphological traits (Figure 2). While our previous controlled-environment study (Devy et al., 2025) demonstrated that drought duration was the dominant driver under high-elevation shade-house conditions (990 m asl), the present field-based investigation shows that soil redox chemistry and mineral availability dominate variation under lowland agroecosystems (~10 m asl).

Despite the apparent abundance of water (Figure 2), growth was constrained under swamp conditions due to acid-sulfate soil toxicity and fluctuating redox dynamics. Plants displayed decreased canopy and stem development but increased palisade mesophyll thickness (Figure 3a), which was consistent with compensatory anatomical adaptations to growth-limiting stress (Fahmi et al., 2018). In contrast to the drought-mediated mechanisms described above (Devy et al., 2025), where growth inhibition was largely due to dehydration stress, repression of growth even under moisture availability suggested Fe^{2+}/Al^{3+}-induced toxicity and oxidative imbalance as major constraints. High Fe and Mn levels (Table 2) are characteristic of redox disequilibrium, reactive oxygen species accumulation, and photosynthetic depression in perennial fruit trees (Pérez-Jiménez and Pérez-Tornero, 2021). The transcript induction of Osmotin (Figure 4) and the metabolic activation of phenylpropanoid biosynthesis (Figure 5c, Table S3) are consistent with an osmotic adjustment and antioxidant defense response described in numerous stress situations (Yepes-Molina et al., 2020; Zandalinas et al., 2022). Consistent with Devy et al. (2025), *Osmotin* activation is an osmotic-stress–related pathway; however, in the current agroecosystem context, it has been linked to ionic imbalance and mineral toxicity rather than to drought-induced dehydration.

Under upland conditions, structural growth prioritization was facilitated by greater uptake of Ca and K (Table 2), nutrients associated with membrane stability and stomatal regulation. Elevated transcription of ABA-responsive genes (*CsNF-YA5*, *CHLASE*; Figure 4), together with flavone accumulation (Figure 5), aligns with moderate stress acclimation and hormonal modulation previously reported in citrus and other woody species (Pereira et al., 2018; Júnior et al., 2026; Buckley, 2019; Li et al., 2025). Rather than identifying novel signaling components, these findings indicate ecological modulation of established ABA–ethylene networks within contrasting soil–water regimes.

Together, these findings indicate that traditional stress-responsive pathways are active, yet their relative contributions and establishment are modulated by agroecosystem-scale drivers. These findings extend previous descriptions of controlled stress responses (Devy et al., 2025) toward insight into mineral-driven ecological reprogramming in the field. These agroecosystem differences were also observed to extend beyond the corresponding transcriptional changes in mineral partitioning.

Specific ionomic profiles were consistently found between agroecosystems: upland-grown plants accumulated more Ca and K, while swamp-grown plants had higher Fe and Mn contents (Table 2). These trends also match the known differences in soil chemistry between aerated mineral soils and acid-sulfate or redox-active swamp soils (Fahmi et al., 2018; Pérez-Jiménez and Pérez-Tornero, 2021; Sulaeman et al., 2024). In contrast to our previous controlled-environment study, in which soil composition was standardized and mineral constraints minimized (Devy et al., 2025), we show here that field-based ionomic variation is a major driver of transcriptional and metabolic responses.

High availability of Fe^{2+} and Mn^{2+} under fluctuating redox conditions may impair membrane integrity, disrupt nutrient uptake balance, and lead to oxidative stress (Pérez-Jiménez and Pérez-Tornero, 2021; He et al., 2023). This positive correlation with the accumulation of Fe/Mn and the up-regulated expression of stress-responsive genes (Figure 4), as well as enrichment in terpenoid- and phenylpropanoid-related metabolites (Figure 5; Table 3), could indicate coordinated antioxidant responses under swamp conditions. Nevertheless, antioxidant metabolism has also been shown to be induced during drought stress (Devy et al., 2025), and the present findings indicate that similar protective mechanisms are triggered by mineral toxicity and hypoxia-associated redox imbalance rather than solely by water deficit.

On the other hand, Ca- and K-enrichment under upland conditions likely facilitated membrane stabilization, stomatal regulation, and enzymatic functions (Brady and Weil, 2017; Hikmat et al., 2022). Calcium participates in secondary signaling pathways and stress perception, whereas potassium is involved in osmotic and turgor regulatory processes (Buckley, 2019; Li et al., 2025). Current results thus build on earlier interpretations to show that mineral nutrient partitioning, rather than drought duration per se, may shape early establishment dynamics in the heterogeneous field setting.

That, early in development, the environmental ionomic signal dominates over genotypic variation, suggests that soil chemistry can exert more than a nominal influence on intrinsic genetic divergence as seedlings establish. Similar tendencies were reported for other perennial crops treated with heterogeneous media (Huang et al., 2025; He et al., 2022). This mineral ecological dominance was not observed in the most homogeneous substrate used by Devy et al. (2025), who emphasized the need to incorporate ionomic profiles into large-scale multi-omics studies under realistic agroecosystem conditions.

Collectively, these results indicate that ionomic reprogramming is a key integrative axis linking soil chemistry with transcriptomic activation, metabolic remodeling, and anatomical adaptation. Rather than identifying novel ion-responsive genes, this study reveals that mineral-driven stress fundamentally reconfigures the relative weighting and coordination of previously described stress pathways across contrasting field environments.

The breeding strategy did not suppress environmental dominance but consistently influenced stress responses, depending on the environment. In comparison to upland, chip-budded canopy development was favored (Figure 2b), and relatively to swamp, bud-assisted marcotting improved establishment performance, associated with PIP1 elevation (Fig. 4).

In contrast to the earlier controlled-environment experiment (Devy et al., 2025), which primarily evaluated responses to drought duration, the present study shows that architectural differences introduced during vegetative propagation influence the magnitude of stress responses under field conditions, consistent with previous findings that propagation method can alter root architecture and whole-plant traits in citrus (Albrecht et al., 2017, 2020).

The marcotting-induced adventitious root system may promote oxygen diffusion and enhance hydraulic buffering capacity under hypoxic conditions (Singh, 2018; Caruso et al., 2020; Habibi et al., 2022). The elevation of aquaporin expression under swamp conditions is a consequence not only of restricted water availability in nature, particularly during extreme drought that season (Zhang et al., 2025), but also of other factors.

This suggests that stress response pathways are not only environmentally but also propagation-induced, and contribute to a genotype-by-environment-by-propagation (G×E×P) interaction framework - the latter not investigated by previous studies.

The propagation strategy also modulated the expression of ethylene-related genes (*ACS2* and *ETR1*). In contrast to Devy et al. (2025), who reported stress-duration-dependent hormonal shifts, the current study indicates that hormonal regulation is architecture-sensitive to field soil heterogeneity.

Upregulation of *Osmotin* and aquaporin genes (*PIP1/PIP2*; Figure 4) coincided with increased accumulation of terpenoid- and phenylpropanoid-related metabolites (Figure 5; Table 3), indicating active osmotic adjustment and antioxidant buffering under stress conditions (Yepes-Molina et al., 2020; Zandalinas et al., 2022; Gui et al., 2024; Fang et al., 2025; Secomandi et al., 2025). Consistent with Devy et al. (2025), these pathways constitute conserved aspects of citrus stress physiology, but here we show, from a field-based analysis, how their coordination and metabolic output are potentially conditioned by soil mineral chemistry and propagation architecture at heterogeneous agroecosystem levels.

The higher accumulation of Fe and Mn specifically under swamp conditions coincided with enhanced phenylpropanoid-related pathways and terpenoid biosynthesis (Table 3), supporting redox-driven metabolic compensation (Pérez-Jiménez and Pérez-Tornero, 2021; Thakur et al., 2022). This pathway results in the synthesis of multiple classes of compounds, including flavonoids, lignin precursors, and phenolic acids that act as reactive oxygen species (ROS) scavengers, UV filters, and structural reinforcement agents under many abiotic stresses (Jia et al., 2022; Li et al., 2024; Cao et al., 2024). A parallel transcriptional and metabolic reprogramming of the phenylpropanoid and terpenoid pathways has been described in woody plants subjected to combined waterlogging and salinity stress (Cisse et al., 2024), supporting the view that secondary metabolism acts as a compensatory mechanism under redox imbalance. Although similar antioxidant induction was observed during progressive drought (Devy et al., 2025), the current findings demonstrate that mineral-induced oxidative disequilibrium can trigger comparable metabolite responses, irrespective of whether it's accompanied by severe drought.

Aquaporin-dependent hydraulic adjustment (*PIP1*/*PIP2*) was simultaneously accompanied by changes in metabolites associated with membrane synthesis and secondary metabolism, indicating that osmotic and structural adjustments are coordinated (Yaneff et al., 2015; Rao et al., 2023; Zhang et al., 2025). Ethylene-related transcription factors (*ACS2*, *ETR1*) also interacted with metabolic signatures linked to stress acclimation (Buckley, 2019; Li et al., 2025). Similar coordinated multilayer regulation of the phenylpropanoid, carotenoid, and terpenoid pathways in response to drought in grapevine has been reported and linked with integrated transcriptional and metabolic reprogramming that improves antioxidant capacity and stress tolerance (Savoi et al., 2016). Compared with our previous controlled drought study

(Devy et al., 2025), which primarily focused on stress-duration-dependent transcriptional changes, the present analysis integrates these interactions within a genotype × environment × propagation (G×E×P) matrix and shows that soil-chemistry heterogeneity and propagation architecture modulate network amplitude and coordination in the field.

The multivariate separation of metabolomic profiles (PLS-DA, PC1 = 32.7%) also demonstrates that agroecosystem context has a structuring influence on biochemical network organization (Figure 5). This indicates that environmental filtering occurs prior to genotype-specific plasticity, modulating the connectivity of metabolic networks early in citrus establishment. Corresponding integrative responses were elicited in woody perennials under compound-stress scenarios (Zandalinas et al., 2022; Rao et al., 2023), but here the integration of propagation architecture and acid-sulfate soil lowland dynamics represents a new ecological dimension that has not been studied to date under controlled hypertonicities.

In general, the current study moves beyond characterizing a controlled drought by establishing a physiologic context for citrus stress within a contextualized field-scale agroecosystem. By contrast to our earlier controlled-environment study (Devy et al., 2025) that focused primarily on duration-of-drought influences under free-ranging soil conditions, passive landform- and water-source-type soil mineral chemistry are shown here to dominate citrus stress responses in heterogeneous lowland agroecosystems. While the molecular components of stress response previously described in citrus remain conserved, their coordination and relative importance are modulated by soil mineral chemistry, hydrological regime, and propagation architecture under contrasting lowland environments. This shift from controlled-stress experiments to field-based ecological interpretation provides new insights into how established stress-response networks operate during early citrus establishment under realistic cultivation conditions.

Practical Implications and Future Directions

The practical implications of these findings for citrus cultivation in marginal tropical agroecosystems are substantial. Building upon the ecological reprogramming framework described above, the results indicate that propagation strategy may be aligned with site-specific soil–water regimes to optimize early establishment and resilience. Under well-drained upland conditions, chip budding promoted greater stem growth and canopy development, particularly in Siam Pontianak, indicating suitability for aerated mineral soils. In lowland tidal-swamp conditions (acid-sulfate soils and variable redox states), bud-assisted marcotting enhanced early growth in Keprok RGL, with an apparent benefit in the root system's adaptation to hypoxic stress.

Instead of finding a propagation method that was superior in all situations, they found that superiority was context-dependent within a genotype × environment × propagation (G×E×P) design. Habitat-related propagation tactics may thus be useful for improving establishment success on degraded or chemically constrained lands, thereby achieving more sustainable intensification of citrus production.

Given the expected increase in the frequency of hydrological extremes, such as recurrent surface waterlogging and long-term drought under climate change (Eckardt et al., 2023), tailoring propagation architecture to local soil–water dynamics may be particularly important for the long-term resilience of orchards. This model should later be extended to older trees, actual performance in terms of nut production, and long-term orchard productivity to confirm the persistence of propagation-induced adaptation across the wide range of tropical cropping systems.

Conclusion

Early establishment of citrus under contrasting lowland agroecosystems is structured by genotype × environment × propagation (G×E×P) interactions, with the environmental water regime and associated soil mineral chemistry exerting a dominant influence during the initial growth stages. Under swamp conditions, mineral-driven redox stress associated with shifts in ion uptake patterns and modulated transcriptional and metabolic coordination, whereas upland environments favored structural growth supported by Ca–K enrichment. The propagation strategy influenced the magnitude and direction of stress-responsive traits in an environment-dependent manner: bud-assisted marcotting was associated with enhanced stress-related responses under swamp conditions, whereas chip budding supported structural growth under upland conditions.

Instead of discovering new molecular pathways, this study shows how the already designated stress-responsive networks function across heterogeneous field environments and under different propagation architectures. These results provide context-dependent guidance for selecting propagation methods across upland and swamp agroecosystems and reinforce the value of multi-omics approaches for assessing early establishment performance in marginal tropical contexts.

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Ethical Approval Statement

We ensure that all research conducted respects participant confidentiality, follows ethical guidelines, and contributes to the academic community with honesty and integrity.

Use of Generative AI Tools Statement

During the preparation of this work, the author(s) used QuillBot, Chat GPT-4, and Claude-3.5sonnet to check grammar and paraphrase. After using this tool/service, the author(s) reviewed and edited the content as needed and take full responsibility for the publication's content.

Contribution of Authors

Triwiratno A & Devy NF: Conceptualized the study, designed the research methodology, and supervised the overall project.

Triwiratno A: Conducted field experiments and contributed to data analysis.

Devy NF: Performed data analysis and prepared the original draft and final manuscript.

Ginting RCB & Purnomo J: Contributed to field establishment, data collection, and resource provision.

Sugiyatno A & Yulianti F: Performed molecular analyses, validated experimental data, and contributed to data visualization.

Hidayah BN & Budiarto K: Conducted mineral analysis and contributed to data curation.

Supriadi K, Ikarini I & Budiyati E: Performed metabolomic and pigment analyses and contributed to methodology development.

Hardiyanto: Contributed to methodology, data validation, formal analysis, and manuscript revision.

All authors read and approved the final draft of the manuscript.

References

Abadie C, Lalande J and Tcherkez G, 2022. Exact mass GC–MS analysis: Protocol, database, advantages and application to plant metabolic profiling. Plant Cell Environ. 45: 3171–3183. https://doi.org/10.1111/pce.14407.

Albrecht U, Bordas M, Lamb B, Meyering B and Bowman KD, 2017. Influence of propagation method on root architecture and other traits of young citrus rootstock plants. HortSci. 52(11): 1569–1576. https://doi.org/10.21273/HORTSCI12320-17.

Albrecht U, Bodaghi S, Meyering B and Bowman KD, 2020. Influence of rootstock propagation method on traits of grafted sweet orange trees. HortSci. 55(5):729-737. https://doi.org/10.21273/HORTSCI14928-20.

Amali N, Fakhrina, Maskartinah, Husni N and Rina Y, 2001. Okulasi-cangkok (okucang) pada tanaman jeruk (A sequential budding–layering propagation system in Citrus Propagation). Banjarbaru: Balai Pengkajian Teknologi Pertanian Kalimantan Selatan, Badan Penelitian dan Pengembangan Pertanian, Kementrian Pertanian, Indonesia.

Auliya I, Hapsari L and Azrianingsih R, 2019. Comparative study of leaf stomata profiles among different ploidy levels and genomic groups of bananas (Musa L.). IOP Conf Ser Earth Environ Sci 391:012037. https://doi.org/10.1088/1755-1315/391/1/012037.

Balfagón D, Segarra-Medina C, Rambla JL and G´omez-Cadenas A, 2025. Metabolic reconfiguration and proline-mediated responses enhance citrus tolerance to combined water, light and heat stress. Plant Stress 18:101039. https://doi.org/10.1016/j.stress.2025.101039.

Brady NC and Weil RR, 2017. The nature and properties of soils. Fifteenth Edition. Pearson, Colombus. USA. ISBN 9780133254488.

Buckley TN, 2019. How do stomata respond to water status? New Phytol. 224: 21–36. https://doi.org/10.1111/nph.15899.

Cao YH, Lü ZL, Li YH, Jiang Y and Zhang JL, 2024. Integrated metabolomic and transcriptomic analysis reveals the role of root phenylpropanoid biosynthesis pathway in the salt tolerance of perennial ryegrass. BMC Plant Biol. 24:1225. https://doi.org/10.1186/s12870-024-05961-1.

Caruso G, Golubkina N, Seredin T, Sellitto VM and Cuciniello A, 2020. Fruit quality and leaf biochemistry of sweet pepper grown under different levels of irrigation and nitrogen fertilization. Agron. 10(12):1920. https://doi.org/10.3390/agronomy10121920.

Cisse EHM, Jiang BH, Yin LY, Miao LF, Zhou JJ, Mekontso FN, Li DD, Xiang LS and Yang F, 2024. Dalbergia odorifera undergoes massive molecular shifts in response to waterlogging combined with salinity. Plant Physiol. 194: 2301–2321. https://doi.org/10.1093/plphys/kiad639.

Dahro B, Li C and Liu JH, 2023. Overlapping responses to multiple abiotic stresses in citrus: from mechanism understanding to genetic improvement. Hortic. Adv. 1:4. https://doi.org/10.1007/s44281-023-00007-2.

Devy NF, Hardiyanto, Budiarti E, Yulianti F, Sugiyatno A, Lenin I, Triwiratno A, Dwiastuti ME, Baswarsiati, Widyaningsih S and Martasari C, 2025. Unraveling gene expression and physio-biochemical responses in citrus species under water stress and water recovery conditions. J. Agric. Food Res. 21: 101898. https://doi.org/10.1016/j.jafr.2025.101898.

Devy, NF, Subandiyah S, Hardiyanto, Yulianti F, Widyaningsih S, Agisimanto D and Dwiastuti ME, 2024. The effect of rootstocks on morphological, physiological, and gene expression characters of citrus seedlings grown under drought conditions. Hortic. Sci. (Prague) 51: 255–269. https://doi.org/10.17221/136/2023-HORTSCI

Eckardt NA, Ainsworth EA, Bahuguna RN, Broadley MR, Busch W, Carpita NC and Pandey S, 2023. Climate change challenges, plant science solutions. Plant Cell 35(1):24-66. https://doi.org/10.1093/plcell/koac303.

Fahmi AI, Nugroho PA, Harsono SS and Adinurani PG, 2018. Relationship of soil chemical properties and plant nutrients content in peat soil under different land uses in Riau Province, Indonesia. Int. J. Sci. Basic Appl. Res. 40(2):21-38.

Fang X, Fan L, Zhou H, Yan H, Ding F, Li R, Linghu Y, Xie B and Wang Y, 2025. Multi-omic analyses reveal the waterlogging induced responses in Magnolia sinostellata. Front. Plant Sci. 16:1653464. https://doi.org/10.3389/fpls.2025.1653464.

Fiehn O, 2017. Metabolomics by Gas Chromatography-Mass Spectrometry: the combination of targeted and untargeted profiling. Curr. Protoc. Mol. Biol. 114: 30.4.1–30.4.32. https://doi.org/10.1002/0471142727.mb3004s114.

Fischer G, Posada FC and Michael Blanke M, 2023. Impact of waterlogging on fruit crops in the era of climate change, with emphasis on tropical and subtropical species: A review. Agron. Colomb. 41(2), 1-15, 2023. https://doi.org/10.15446/agron.colomb.v41n2.108351.

Gui G, Zhang Q, Hu W and Liu F, 2024. Application of multiomics analysis to plant flooding response. Front. Plant Sci. 15:1389379. https://doi.org/10.3389/fpls.2024.1389379.

Habibi F, Ramezanian A, Rahemi M, Eshghi S, Guillén F, Serrano M and Valero D, 2022. Physiological, biochemical, and molecular responses of fruit trees to root zone hypoxia. Environ. Exp. Bot. 206:105179. https://doi.org/10.1016/j.envexpbot.2022.105179.

Hanlon EA, Morgan KT, Obreza TA and Mylavarapu R, 2012. Leaf Analysis in Citrus: Developments in Analytical Techniques. In: Srivastava, A. (eds) Advances in Citrus Nutrition. Springer, Dordrecht. https://doi.org/10.1007/978-94-007-4171-3_6.

He W, Xie R, Wang Y, Chen Q, Wang H, Yang S and Wang X, 2022. Comparative transcriptomic analysis on compatible/incompatible grafts in *Citrus*. Hort. Res. 9: uhab072. https://doi.org/10.1093/hr/uhab072.

He W, Luo L, Xie R, Chai J, Wang H, Wang Y, Chen Q, Wu Z, Yang S, Li M, Lin Y, Zhang Y, Luo Y, Zhang Y, Tang H and Wang X, 2023. Transcriptome sequencing analyses uncover mechanisms of citrus rootstock seedlings under waterlogging stress. Front. Plant Sci. 14:1198930. https://doi.org/10.3389/fpls.2023.1198930.

Hikmat M, Hati DP, Pratamaningsih MM and Sukarman, 2022. High productivity dry land in Nusa Tenggara for agricultural development. Jurnal Sumberdaya Lahan Vol. 16 No. 2, Desember

2022: 119-133. http://dx.doi.org/10.21082/jsdl.v16n2.2022.119-133.

Huang D, Li J, Wei Y, Xie X, Qin Z, Chen J and Liu Y, 2025. Integrating multi-omics analysis unravels the molecular mechanisms underlying variations in bioactive flavonoids in *Citrus reticulata* 'Chachi' grafted onto different citrus rootstocks. Ind. Crops Prod. 232:121287. https://doi.org/10.1016/j.indcrop.2024.121287

Jia C, Guo B, Wang B, Li X, Yang T, Li N and Guo X, 2022. Integrated metabolomic and transcriptomic analysis reveals the role of phenylpropanoid biosynthesis pathway in tomato roots during salt stress. Front. Plant Sci. 13:1023696. https://doi.org/10.3389/fpls.2022.1023696

Júnior AW, Ratcliffe RG, Lee PJ and Sweetlove LJ, 2026. Metabolic regulation under oxygen deprivation and reoxygenation in plants. New Phytol. 231(1):20-35. https://doi.org/10.1111/nph.17821

Kunarso A, Bonner MTL, Blanch EW and Grover S, 2022. Differences in Tropical Peat Soil Physical and Chemical Properties. J. Soil Sci. Plant Nut. https://doi.org/10.1007/s42729-022-01008-2

Li C, Wang P, Xia Y, Zhang Z, Yang Z, He M, Kang K, Jiang N, Rong X and Yang L, 2025. Ethylene-mediated integration of metabolic regulation and stomatal closure for enhanced waterlogging tolerance in *Brassica napus* L. Ind. Crops Prod. 235: 121807. https://doi.org/10.1016/j.indcrop.2025.121807.

Li W, Lin YCJ, Chen YL, Zhou C, Li S, Ridder ND, Oliveira DM, Zhang L, Zhang B, Wang JP, Xu CZ, Fu X, Luo K, Wu AM, Demura T, Lu MZ, Zhou Y, Li L, Umezawa T, Boerjan W and Chiang VL, 2024. Woody plant cell walls: Fundamentals and utilization. Mol. Plant 17: 112–140. https://doi.org/10.1016/j.molp.2023.12.008.

Martínez-Cuenca MR, Forner-Giner MA, Iglesias DJ, Primo-Millo E and Legaz F, 2015. Flooding impairs Fe uptake and distribution in *Citrus* due to the strong down-regulation of genes involved in Strategy I responses to Fe deficiency in roots. PLoS One 10(4): e0123644. https://doi.org/10.1371/journal.pone.0123644

Metusala D, 2017. An alternative simple method for preparing and preserving cross-section of leaves and roots in herbaceous plants: Case study in *Orchidacea*e. AIP Conf Proc 1862:030113. https://doi.org/10.1063/1.4991217

Misra BB, Langefeld C, Olivier M and Cox L A, 2019. Integrated omics: tools, advances and future approaches. *J. Mol. Endocrinol.* 62(1): R21–R45. https://doi.org/10.1530/JME-18-0055

Nelson DW and Sommers LE, 1996. Total carbon, organic carbon, and organic matter. In *Methods of Soil Analysis, Part 3: Chemical Methods*, D.L. Sparks, ed. (Madison, WI: SSSA), pp. 961–1010.

Noor M, Sukarman, Masganti, Hairani A, Khairullah I and Alwi M, 2022. Fifty-three years' research and development of swampland for food production. J. Sumberdaya Lahan 16 (2): 111-118. http://dx.doi.org/10.21082/jsdl.v16n2.2022.111-118.

Öhlinger R, Kandeler E, Gerzabek M, Insam H and Illmer P, 1996. Methods in Soil Chemistry. In: Schinner, F., Öhlinger, R., Kandeler, E., Margesin, R. (eds) Methods in Soil Biology. Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-642-60966-4_29

Pereira L, Barros L, Carvalho AM and Ferreira IC, 2018. Use of UFLC-PDA for the analysis of organic acids in thirty-five species of food and medicinal plants. Food Analyt. Methods 11:2871-2879. https://doi.org/10.1007/s12161-018-1260-2.

Pérez-Jiménez M and Pérez-Tornero O, 2021. Iron deficiency and excess affect growth and biological responses in *Hypericum perforatum* L. plants. J. Soil Sc. Plant Nutr. 21:197-211. https://doi.org/10.1007/s42729-020-00352-9

Rao MJ, Feng B, Ahmad MH, Tahir ul Qamar M, Aslam MZ, Khalid MF, Hussain S, Zhong R, Ali Q, Xu Q, Ma C and Wang L, 2023. LC-MS/MS-based metabolomics approach identified novel antioxidant flavonoids associated with drought tolerance in citrus species. Front. Plant Sci. 14:1150854. https://doi.org/10.3389/fpls.2023.1150854

Ritung S, NugrohoK., Mulyani A and Sukarman, 2011. Technical Guidelines for Land Evaluation for Agricultural Commodities. Indonesian Center for Agricultural Land Resources Research and Development, Bogor, Indonesia.

Salem MA, Takuya Y, de Souza LP, Saleh A, Krzysztof B, Alisdair RF and Patrick G, 2020. An improved extraction method enables the comprehensive analysis of lipids, proteins, metabolites and phytohormones from a single sample of leaf tissue under water-deficit stress. The Plant J. 103:1614–1632. https://doi.org/10.1111/tpj.14800.

Savoi S, Wong DCJ, Arapitsas P, Miculan M, Bucchetti B and Peterlunger E, 2016. Transcriptome and metabolite profiling reveals that prolonged drought modulates the phenylpropanoid and terpenoid pathway in white grapes (*Vitis vinifera* L.). BMC Plant Biol. 16:67. https://doi.org/10.1186/s12870-016-0760-1.

Secomandi E, Palmioli A, Gerna D, Kuang L, Airoldi CA and Pennisi G, 2025. Multi-omics approaches to studying waterlogging stress responses in *Arabidopsis thaliana*: Insights into metabolic reprogramming. Plant Cell Environ. 48(1):68-85. https://doi.org/10.1111/pce.14762.

Singh J, 2018. Mechanism of plant hormone signaling under stress. Frontiers in Plant Sci. 9:1-19. https://doi.org/10.3389/fpls.2018.00473.

Sulaeman Y, Maftuáh E, Noor M, Hairani A, Nurzakiah S, Mukhlis M, Anwar K, Fahmi A, Saleh M, Khairullah I, Rumanti IA, Alwi M, Noor A and Ningsih RD, 2024. Coastal Acid-Sulfate Soils of Kalimantan, Indonesia, for Food Security: Characteristics, Management, and Future Directions. Resources 13:36. https://doi.org/10.3390/resources13030036

Thakur M, Praveen S, Divte PR, Mitra R, Kumar M, Gupta CK and Kumar RR, 2022. Metal tolerance in plants: Molecular and physicochemical interface determines the not so heavy effect of heavy metals. *Chemosphere* 287:131957. https://doi.org/10.1016/j.chemosphere.2021.131957.

Wishart DS, 2019. Metabolomics for investigating physiological and pathophysiological processes. Physiol Rev. 99: 1819–1875. DOI: https://doi.org/10.1152/physrev.00035.2018.

Yaneff A, Vitali V and Amodeo G, 2015. PIP1 aquaporins: Intrinsic water channels or PIP2 aquaporin modulators? FEBS Letters 589: 3508–3515. http://dx.doi.org/10.1016/j.febslet.2015.10.018

Yang N, Gong Z, Li Z, Zheng J, Liu Z, Wang B, Geng S, Sun F, Chen H, Li S, Wang J and Liang Y, 2025. Multiomic analyses reveal transcription factors involved in the fatty acid biosynthesis pathway under cold stress in upland cotton (*Gossypium hirsutum*). Front. Plant Sci. 16:1733102. http://doi.org/10.3389/fpls.2025.1733102.

Yepes-Molina L, Martínez-Ballesta MC and Carvajal M, 2020. Plant plasma membrane vesicles: A good strategy to gain insight into the role of aquaporins in plant tolerance to osmotic and heavy metal stress. Environ. Exp. Bot. 171:103918. https://doi.org/10.1016/j.envexpbot.2019.103918

Yulianti F, Adiredjo AL, Soetopo L and Ashari S, 2021. Changes in physiological and relative genes expression response of Mandarin citrus (*Citrus reticulata* Blanco) cv Rimau Gerga Lebong (RGL) grafted onto different citrus rootstocks, Indian J. Agric. Res. I: 549–555. https://doi.org/10.18805/IJARe.A-615.

Zandalinas SI, Sales C, Beltrán J, Gómez-Cadenas A and Arbona V, 2017. Activation of Secondary Metabolism in Citrus Plants Is Associated to Sensitivity to Combined Drought and High Temperatures. Front. Plant Sci. 7:1954. https://doi.org/10.3389/fpls.2016.01954.

Zandalinas SI, Balfagón D, Gómez-CadenasA and Mittler R, 2022. Plant responses to climate change: metabolic changes under combined abiotic stresses. J. Exp. Bot. 73 (11):3339–3354. https://doi.org/10.1093/jxb/erac073.

Zhang M, Liu W, Wang C, Lin S, Chen Y, Cui H, Xiang C, Ma Y, Li X, Lu Y, Han X, Dong Y, Gao L and Zhang W, 2025. Root-to-shoot mobile mRNA CmoKARI1 promotes JA-Ile biosynthesis to confer chilling tolerance in grafted cucumbers. Nat. Commun. 16: 7782. https://doi.org/10.1038/s41467-025-63228-1